



⑫

EUROPEAN PATENT APPLICATION

⑬ Application number: 92119498.1

⑮ Int. Cl. 5: C07K 3/00, A61K 9/16,
C07K 3/24

⑯ Date of filing: 13.11.92

⑰ Priority: 14.11.91 US 792292

⑲ Date of publication of application:
19.05.93 Bulletin 93/20

⑳ Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LU MC
NL PT SE

㉑ Applicant: THE TRUSTEES OF PRINCETON
UNIVERSITY
Nassau Street
Princeton New Jersey 08544(US)

㉒ Inventor: Debenedetti, Pablo G.
32 Clover Lane
Princeton, New Jersey 08544(US)

Inventor: Lim, Goo-Bin
2-1101 Hanyang Apartment,
Apgujung-dong
Kangnam-gu, Seoul(KR)

Inventor: Lundström, Robert K.

61 Lillie Street

Princeton University, New Jersey 08544(US)

㉓ Representative: Minoja, Fabrizio
Studio Consulenza Brevettuale Via Rossini,
8
I-20122 Milano (IT)

㉔ Formation of protein microparticles by antisolvent precipitation.

㉕ A method of forming microparticles of a material which comprises bringing a supercritical antisolvent gas into contact with a solution of said material in a solvent at a controlled rate operable to expand the solution and precipitate the material.

The present invention relates to a method of forming protein microparticles using a nonsolvent precipitation and to compositions of such proteins.

Conventional means of administering drugs (e.g., pills and tablets) provide a single burst or peak of drug in the blood. This initial spike is followed by a decay in blood concentration. Because every drug has a range of concentration below which its therapeutic effect is limited, and above which toxic side effects occur, it is desirable to release the drug at a controlled rate and minimize fluctuations. In controlled release, this is achieved by incorporating a rate-limiting step into the design of the delivery system. Among the many types of controlled release systems there are bioerodible polymer microspheres in the range of 1 to 50 micrometers (μm). Such small microspheres can be injected subcutaneously or intramuscularly. Bioerodible polymers are materials that are degraded by body fluids to non-toxic products. The polymer particles contain the drug of interest in dispersed form. Drug release occurs partly as a result of polymer degradation inside the body. Systems aimed at providing spatial or temporal control of drug release in the body are referred to generically as controlled drug delivery devices.

Controlled release of proteins, such as therapeutic enzymes, requires the formation of small particles which can be uniformly dispersed in the polymer matrix. Techniques to produce protein particles include spray drying, lyophilization, milling, grinding, and protein micronisation, WO/90/132. Only the last method leads to small particles.

Jean W. Tom and Pablo G. Debenedetti, "Formation of Bioerodible Microspheres and Microparticles by Rapid Expansion of Supercritical Solutions", Department of Chemical Engineering, Princeton University, 1991, disclose a process to make biocompatible and bioerodible polymer microspheres, mainly polyhydroxy acids including poly(L-lactic acid) (L-PLA), poly(D,L-lactic acid), (DL-PLA) and poly(glycolic acid) (PGA). Microparticles and microspheres of these polymers were made with the goal of being used for controlled delivery of pharmaceuticals. Nucleation of poly(L-lactic acid) from CO_2 and CO_2 -acetone mixtures produced microparticles and microspheres ranging from 4 to 25 micrometers (μm). Microspheres (2-20 μm) were also obtained using chlorotrifluoromethane as a solvent.

The technique to produce the microspheres and microparticles used by Tom and Debenedetti involved applying rapid expansion of supercritical solutions. This was known from Matson *et al.*, "Expansion of Supercritical Fluid Solutions: Solute Formation of Powders, Thin Films and Fibers", *Ind. Eng. Chem. Res.* 26, 2298-2306 (1987). In the

process of rapid expansion of supercritical solutions, a nonvolatile solute is dissolved in a supercritical fluid. The resulting solution is highly compressible in the vicinity of the solvents critical point. Nucleation of the solute is triggered mechanically by reducing the solution's density through a rapid expansion, thereby reducing its dissolving capacity; Kumar *et al.*, "Modelling the Solubility of Solids in Supercritical Fluids with Density as the Independent Variable", *J. Supercrit. Fluids*, 1988, 1, 15-22. The combination of a rapidly propagating mechanical perturbation and high supersaturation ratios leads to uniform conditions within the carrier fluid and hence, in principle, to narrow particle size distributions into small particles.

Chang *et al.*, "Solvent Expansion and Solute Solubility Predictions in Gas-Expanded Liquids", *AIChE Journal*, 36, No. 6, 939-942 (1990) disclose gas antisolvent addition for liquid phase precipitation of solids. See also Gallagher *et al.*, "Gas (Gas-Anti-Solvent) Recrystallization: A New Process to Recrystallize Compounds Insoluble in Supercritical Fluids", *Am. Chem. Soc. Symp. Ser.*, No. 406 (1989).

Chang *et al.* disclose recrystallization of ac-statinophen from butanol and β -carotene from toluene using CO_2 . The CO_2 was charged at the top of the column or reservoir containing the solution to be gas expanded.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing of an experimental apparatus for the gas antisolvent recrystallization and liquid expansion useful in the present invention. In Figure 1 "V" represents valves, "PI" represents pressure sensors, "TI" represents temperature sensors. Back pressure regulators, rotameters, filters, check valves, metering valves, shut-off valves, rupture discs, and heat exchangers are depicted with conventional symbols.

Figure 2 is a microphotograph magnified 8,000 times showing catalase particles made in Example 1 the particles collected on the glass slides upside.

Figure 3 is a microphotograph of the same material as in Figure 2 having a magnification of 15,000.

Figure 4 is a microphotograph of the same material as in Figure 3 where the particles are collected on a filter and magnified 10,000 times.

Figure 5 is a microphotograph as recited in Figure 4 magnified 15,000 times.

Figure 6 is a microphotograph of the insulin particles made according to Example 2 magnified 10,000 times and collected on a filter.

Figure 7 is a microphotograph of insulin particles made in Example 2 magnified 5,500 times and collected on a filter.

Description

Referring to Figure 1, a solution of dissolved material, preferably a protein solution, from solution source 14 (in which the solution can be prepared and/or stored) is fed to crystallizer 10. The solution is fed through suitable flow metering means, such as rotameter 30 and high pressure liquid pump 32. The pressure can be controlled using a back-pressure regulator 34. The solution can be brought to the desired temperature by a suitable heating means, such as coils 36, which are kept at the desired temperature by circulating air. Heating can be provided by strip heaters and forced circulation of air.

The pressurized solution is fed to crystallizer chamber 20. Preferably, it is injected into the top of the crystallizer through a laser-drilled platinum disc 53 to produce a fine spray of solution droplets in the crystallizer chamber 20. Disc 53 thus will have at least one orifice to produce a fine spray. Typical the orifice is from 5 to 50 and preferably from 10 to 30 and most preferably from 15 to 20 micrometers in diameter. Preferably the solution takes the form of a plurality of droplets having a diameter of from 10 to 500 μm , at least one continuous fine stream having a diameter of less than 1 millimeter, or a thin film having a thickness of less than 1 millimeter.

An antisolvent gas is fed from antisolvent gas tank 18 to an antisolvent gas compression pump 40. The gas pressure can be controlled by a gas back-pressure regulator 42. The temperature of the antisolvent in tank 18 will range of from 10 to 40°C, preferably from 20 to 30°C, such as liquid carbon dioxide at 25°C. The antisolvent is cooled in heat exchanger 44 with solvent pump 40 bringing the liquid antisolvent to supercritical pressure. Excess solvent in the gas stream can recycle back to the liquid inlet side of the pump 40.

Typical conditions at the outlet of pump 40 are from 25 to 45°C and more preferably from 30 to 40°C, and 60 to 200 atmosphere pressure, more preferably 100 to 150 atmosphere pressure. The compressed antisolvent is fed through suitable micrometering means, such as micrometering valve 46. Optionally, there can be additional thermostating means such as coils 48 between solvent pump 40 and crystallizer 10. The supercritical antisolvent gas then is fed to crystallizer chamber 20 at a controlled rate so as to contain a continuum of supercritical gas.

In crystallizer chamber 20 the supercritical antisolvent gas dissolves in the protein solution at a controlled rate depending on the stream or droplet geometry, temperature and concentration. As solution expands, the soluble material precipitates out. Use of a continuum of supercritical fluid and

passage of a fine spray, film, or droplets through the supercritical gas results in rapid expansion of the liquid solution and precipitation of the dissolved material as extremely fine particles less than 10 micrometers in equivalent diameter and preferably less than 5, more preferably less than 3, and most preferably less than 1 micrometer in diameter, particularly for soluble material which precipitates out in a globular shape. Needle-like precipitates have a diameter of less than about 3 and more preferably less than 1 micrometer with a length of less than 5, and preferably less than 3 micrometers. The rapid precipitation results in a narrow particle distribution as exemplified and shown in Figures 2 to 7.

The depleted solution and spent supercritical antisolvent gas are fed to depressurization tank 22, to be brought back to ambient conditions. The precipitated crystals are collected from the crystallizer 10 at crystal collection port 26. The crystals can be collected by any suitable means, such as on a filter and/or a glass plate.

A fluid mixture of spent solvent and supercritical fluid can be collected from the bottom of the crystallizer chamber 20 through line 50. The fluid mixture passes through valve V7 to collection tank 52 through valve V4 to depressurization tank 22 where the mixture is depressurized and expanded to atmospheric pressure.

The system should be sized to handle pressures of up to 6000 psi, and preferably in the range of from atmospheric pressure to 6,000 psi from temperatures ranging from 20°C to 60°C and preferably 30°C to 50°C.

Different flow patterns can be used in crystallizer 10. The direction of antisolvent supercritical gas flow in the crystallizer (upward or downward) can be determined by the valves before and after the crystallizer. For upward flow in the crystallizer, valve V2 and valve V3 are open, and valve V1 and valve V4 are closed. Where the antisolvent fluid is to flow downward the valves are reversed. The flow of protein solution can be cocurrent or countercurrent to the flow of antisolvent fluid. In the preferred embodiment with continuous operation, protein solution is pumped into the crystallizer by high pressure liquid pump 32 and its instantaneous flow rate is measured by the liquid rotameter 30. The pressure is controlled using a backpressure regulator 34 and pressurized protein solution is injected into the top of the crystallizer. The antisolvent gas is also injected into the top of the crystallizer for cocurrent flow of both the supercritical solvent fluid continuum through the crystallizer and the protein solution through the crystallizer, both from top to bottom. The crystallizer can be operated in batch, semi-batch or continuous operation. The solution of soluble material can be passed through cocur-

rently or countercurrently in relation to a continuum stream of antisolvent supercritical gas.

Suitable soluble material are protein, particularly hydrophobic enzymes such as insulin, catalase, adrenocorticotrophin hormone, and peroxidase. The method has applicability, however, to virtually any protein and is not dependent on chemical structure or biological activity.

Useful solutions for the protein comprise at least one non-aqueous solvent suchs as ethanol, formamide, dimethylsulfoxide, tetrahydrofuran, acetic acid, dimethylformamide, ethylene glycol, liquid polyethylene glycol and dimethylaniline.

Supercritical gases which can be used include (with an indication of its critical temperatures (C) and critical pressures (atm)) include ethane (32.2C, 48.1 atm), ethylene (9.21C, 39.7 atm); sulfur hexafluoride (45.5C, 37.1 atm), nitrous oxide (36.5C, 71.7 atm) chlorotrifluoromethane (28C, 38.7 atm), and monofluoromethane (44.5C, 58 atm). A solution of water and ethanol has been used. However, the presence of water in such solutions has been found to lower the production of small particle protein.

In accordance with the present invention there is obtained a protein composition having protein particles wherein substantially all of the protein particles are artificially isolated and have an equivalent diameter of less than 5, more preferably less than 3, and most preferably less than 1 micrometer. The protein composition has a narrow particle distribution shown in Figures 2-7. These proteins have uniform or controlled chemical compositions. Therefore, samples of a composition consisting essentially of a desired protein can be isolated and made.

The isolated proteins of the present invention can be used to make temporal drug release compositions. Such compositions can comprise a bioerodible polymeric matrix and at least one protein having an equivalent diameter of less than 3 micrometers. Preferred polymers are polyhydroxy acids such as those selected from the group consisting of poly(L-lactic acid), poly(D,L-lactic acid) and poly(glycolic acid). The composition can comprise for 0.1 to 50 weight percent of the protein.

Preferably the drug release compositions contain a polymer matrix having a continuum of bioerodible polymer matrix with the protein particles dispersed therewith. Such particles can be made by means known in the art as discussed above.

Following are several Examples which illustrate the nature of the invention and the manner of carrying it out.

Referring to Figure 1, liquid carbon dioxide in solvent tank 18 was compressed by high pressure

liquid pump 40 which was an American Lewa Plunger Metering Pump Model EL-1; rated at 6,000 psi and 2 gallons per hour. The pressure was controlled by a back-pressure regulator 42 which was a Tescom, Model 54-2100 Series, rated at 6,000 psi. The compressed carbon dioxide was introduced into a see-through crystallizer 20 which was a Jerguson Gauge, Model 19T40, 316 stainless steel 5,000 psi, 1.3 centimeter by 1.3 centimeter, 31.8 centimeter long, 50 cubic centimeter through micrometering valve 46 which was an Autoclave Engineering Micrometering Valve 60VRMM. The pressurized carbon dioxide was preheated in coiled tubes 48.

The pressure in the crystallizer was indicated by a crystallizer pressure gauge 54 which was a Bourdon Gauge, Omega Model PGJ-45B-5000, rated at 5000 psi, and controlled by back-pressure regulator 59 which was a Tescom 26-1700 Series, rated at 6,000 psi.

The protein solution from protein solution tank 14 was pumped in continuous operation by a high pressure pump which was a Milton Roy LDC Duplex Metering Pump. The instantaneous flow was measured by liquid rotameter 30 which was a Fischer and Porter; Model 10A6132, 0-14 cubic centimeters per minute of water flow. The pressure of the protein solution was controlled using a back-pressure regulator 34 which was a Tescom; 26-1700 Series, 10,000 psi rated regulator. The protein solution was preheated in coiled tubes 36.

The pressurized protein solution was injected into the top of the crystallizer 10 through a laser-drilled platinum disc 53, Ted Pella; 3 mm OD x 0.24 mm thick; 20 micrometers in diameter.

At the bottom of the crystallizer the protein particles were precipitated and deposited on an inclined glass slide after crystallization. The plane of the glass slide 55 was at a 10° angle to the direction of the protein solution flow. Additionally, a filter 57, Mott Metallurgical; 316 Stainless Steel 1.6 centimeters in diameter, 0.5-2 micrometer pore size was located below the glass slide to collect all the protein particles. A thermocouple 56, Omega Engineering Type J, was placed in the middle of the crystallizer to monitor the temperature.

Protein particles collected on the glass slides were examined through a Carl Zeiss Universal Optical Microscope and a Scanning Electron Microscopy JEOL JSM-840A, with samples coated with gold-palladium. The particles on the microfilter were also examined with the Scanning Electron Microscope.

The fluid mixture of carbon dioxide, ethanol and water coming out of the crystallizer was depressurized and expanded to atmospheric pressure by passing through a cylindrical depressurizing tank 22, Swagelok, 150 ml, 5,000 psi and back-

pressure regulator 58, Tescom, 1700 Series, rated at 6,000 psi.

The instantaneous and total flow rates of solute free CO₂ gas were measured with rotameter 60 (Fischer and Porter; Model 10A4555, 0-3.35 SCFM AIR and dry test meter 62, American Meter; Model DTM200A, respectively).

During the experiment the normal flow rates of protein solution and antisolvent gas were 0.35 cm³/min and 35g/min, respectively, and typical operating time was 4 hours for continuous operation. The whole system was enclosed in an air chamber where temperature was controlled using a PID temperature controller, Omega Engineering Model CN9000, and strip heaters.

To measure the expansion behavior of CO₂ - ethanol solution, 20 mls of ethanol solution was preloaded into the crystallizer and pressure was increased by 200 psi increments through valves V2 and V7. Gas solvent was then circulated through valve V8, crystallizer 20 and valve V5 using a high pressure compressor (Haskell; Double Acting Single Stage Model ACD-62) with closed valves V6 and V7 until the system reached the equilibrium state and the liquid level remained constant.

Example 1

Catalase particles, Figures 2-5, were made having an equivalent diameter of less than 1 μm.

A solution of 20 mg catalase (from bovine liver) [Sigma Chemicals C-40] in 200 ml of 90% ethanol (Pharmco Products Co., 200 proof) and 10% water (deionized through a reverse osmosis apparatus, Hydro Picosystem) was used. The pH of the solution was adjusted to 3.22 with hydrochloric acid.

Liquid carbon dioxide (MG industries; Bone-dry grade, >99.8%) was compressed by a high pressure liquid pump 40. The delivery pressure (1600 psi) was controlled by a back-pressure regulator 42. The pressurized liquid was preheated to a supercritical temperature (35°C) and flowed through coiled tube 48 before entering the crystallizer 10. The system was enclosed and thermostating was achieved by circulating hot air under temperature control (Omega Engineering Model CN9000), with heating provided by strip heaters. The see-through crystallizer chamber 20 was kept at 35°C.

The supercritical fluid was fed to the crystallizer through a micrometering valve 46, with valves V1 and V4 open; and V2 and V3 closed. The pressure inside the crystallizer was kept at 1300 psi by back-pressure regulator 59. The instantaneous and total flow rates of supercritical fluid were measured with rotameter 60 and dry test meter 62, respectively. The flow rate of the supercritical fluid

was 33 g/min.

The liquid solution containing the enzyme was pressurized and circulated by liquid pump 32 and back-pressure regulator control (1430-1530 psi). The solution circulated through coil 36 and was preheated to 35°C. It entered the top of the crystallizer through a laser-drilled platinum disc (Ted Pella: 3 mm OD x 0.24 mm thick; 20 μm), and emerged as very small droplets. The liquid flow rate was 0.35 cc/min. The liquid and supercritical streams circulated cocurrently downwards.

The fluid mixture of carbon dioxide, ethanol and water exiting the crystallizer was depressurized and expanded to atmospheric pressure by flowing through cylindrical depressurizing tank 22, and back-pressure regulator 58.

The supercritical fluid expanded and eventually dissolved most of the liquid solvent, causing the enzyme particles to precipitate. The particles were collected on an inclined glass slide located at the bottom of the crystallizer, forming an angle of approximately 10° to the direction of the protein solution's flow. Particles were also collected on a filter (Mott Metallurgical; 316 Stainless Steel, 1.6 cm diameter, 0.5 μm pore size). The carbon dioxide outlet was located approximately 8 cm above the filter. The duration of the experiment was 260 minutes.

Figures 2 and 3 are particles collected on the glass slide's up side (facing the nozzle). Figures 4 and 5 are particles collected on the filter.

Example 2

The pH of a solution of 20 mg zinc insulin [Miles; low endotoxin 86-003] in 200 ml of 90% ethanol (Pharmco Products Co., 200 proof)-10% water (deionized through a reverse osmosis apparatus, Hydro Picosystem) was adjusted to 2.56 with hydrochloric acid.

Liquid carbon dioxide (MG industries; Bone-dry grade, >99.8%) was compressed by a high pressure liquid pump 40. The delivery pressure (2000 psi) was controlled by a back-pressure regulator 42. The pressurized liquid was preheated to a supercritical temperature (35°C) as it flowed through coiled tube 48 before entering the crystallizer 10. The system was enclosed, and thermostating was achieved by circulating hot air under temperature control (Omega Engineering Model CN9000), with heating provided by strip heaters. The see-through crystallizer chamber 20 was kept at 35°C.

The supercritical fluid was fed to the crystallizer through a micrometering valve 46, with valves 1 and 4 open; and 2 and 3 closed. The pressure inside the crystallizer was kept at 1300 psi by back-pressure regulator 59. The instantaneous

and total flow rates of supercritical fluid were measured with a rotameter 60 and a test meter 62, respectively. The flow rate of the supercritical fluid was 35.6 g/min.

The liquid solution containing the enzyme was pressurized and circulated by liquid pump 32 under back-pressure regulator 34 control (1450 psi). The solution circulated through coil 36 and was preheated to 35°C. It entered the top of the crystallizer through a laser-drilled platinum disc (Ted Pella; 3 mm OD x 0.24 mm thick; 20 μm), and emerged as very small droplets. The liquid flow rate was 0.39 cc/min. The liquid and supercritical streams circulated cocurrently downwards.

The fluid mixture of carbon dioxide and water exiting the crystallizer was depressurized and expanded to atmospheric pressure by flowing through cylindrical depressurizing tank 22 and a back-pressure regulator 58.

The supercritical fluid expanded and eventually dissolved most of the liquid solvent, causing the enzyme particles to precipitate. The particles were collected on an inclined glass slide located at the bottom of the crystallizer, forming an angle of approximately 10° to the direction of the protein solution's flow. Particles were also collected on a filter (Mott Metallurgical; 316 Stainless Steel, 1.6 cm diameter, 0.5 μm pore size). The carbon dioxide outlet was located approximately 60 mm below the filter.

The duration of the experiment was 296 minutes for carbon dioxide input, and 237 minutes for liquid input, followed by 17 minutes of liquid solution flow without dissolved enzyme.

Figures 6 illustrates particles collected on the filter which were needlelike having a diameter of less than 1 μm and being less than 3 μm . Figure 7 illustrates particles collected which were globular, having an equivalent diameter of less than about 1 μm .

While exemplary embodiments of this invention have been described, the true scope of the invention is determined from the following claims.

Claims

1. A method of forming microparticles of a material which comprises bringing a supercritical antisolvent gas into contact with a solution of said material in a solvent at a controlled rate operable to expand the solution and precipitate the material.
2. The method of claim 1 wherein the material is a protein.
3. The method of claim 2 wherein the protein is a hydrophobic enzyme.

4. The method of claim 2 wherein the protein is selected from the group consisting of insulin, catalase, adrenocorticotrophin hormone and peroxidase.

5. The method of claim 2 wherein the protein solvent includes at least one of ethanol, dimethylsulfoxide, tetrahydrofuran, acetic acid, formamide, dimethyl-formamide, ethylene glycol, liquid polyethylene glycol and dimethylaniline.

10 6. The method of claim 2 wherein there is at least one antisolvent gas includes at least one of carbon dioxide, ethane, ethylene, sulfur hexafluoride, nitrous oxide, chlorotrifluoromethane and monofluoromethane.

15 7. The method of claim 2 wherein the antisolvent gas is added at a rate sufficient to precipitate substantially all of the particles at a particle size of less than 5 μm equivalent diameter.

20 8. The method of claim 7 wherein the protein forms generally globular particles having a equivalent diameter of less than 1 μm .

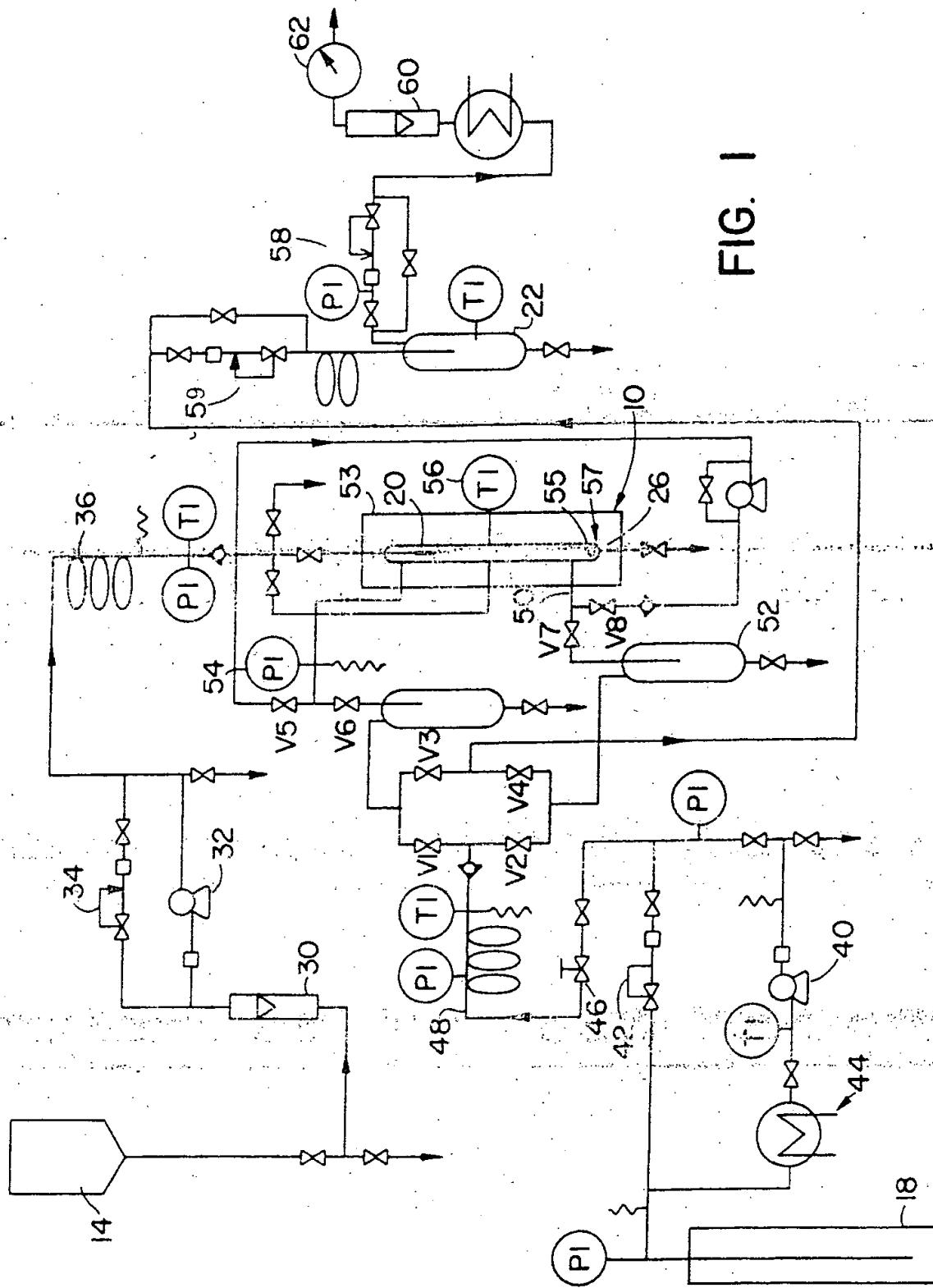
25 9. The method of claim 7 wherein the protein forms needle-like particles having an average diameter of less than 1 μm and a length of less than 3 μm .

30 10. The method of claim 1 wherein solution is in the form of droplets.

35 11. The method of claim 1 comprising continuously passing a plurality of droplets cocurrently with a stream of antisolvent gas.

40 12. The method of claim 1 comprising continuously passing a plurality of droplets counter-currently with a stream of antisolvent gas.

FIG. 1



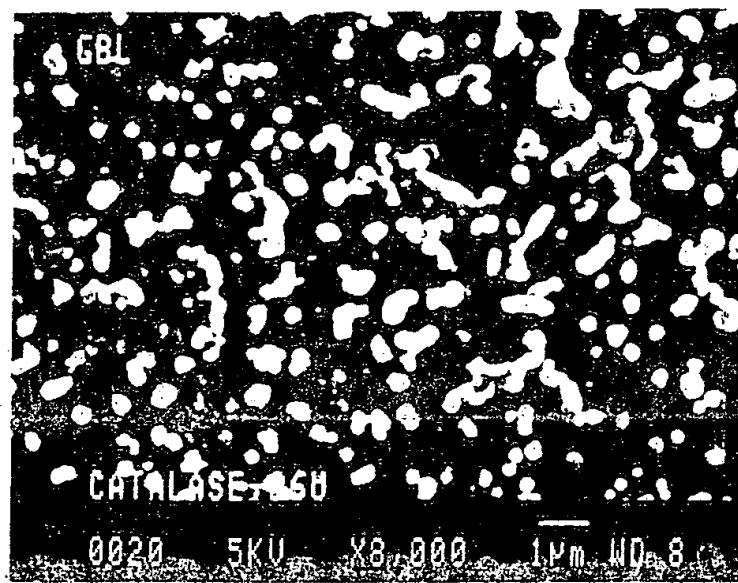


FIG. 2

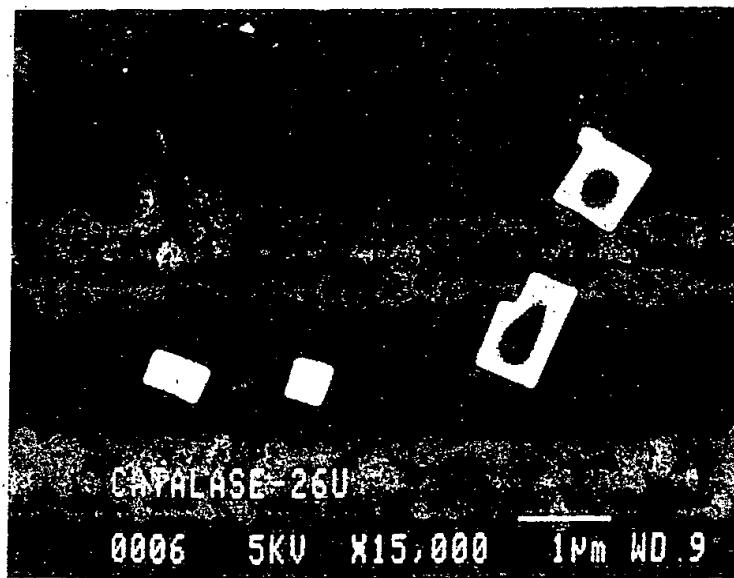


FIG. 3

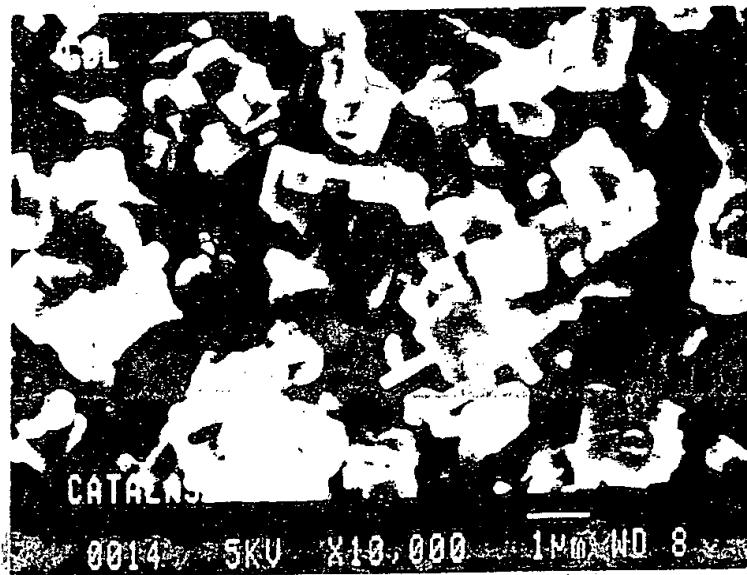


FIG. 4

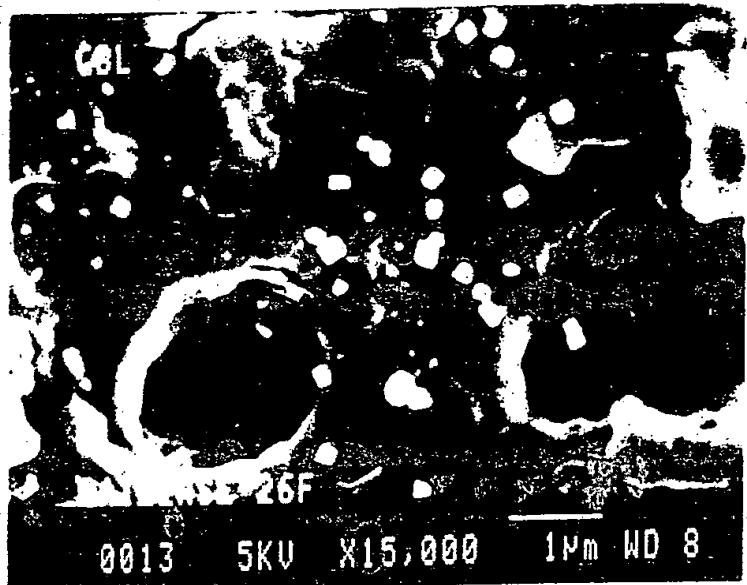


FIG. 5

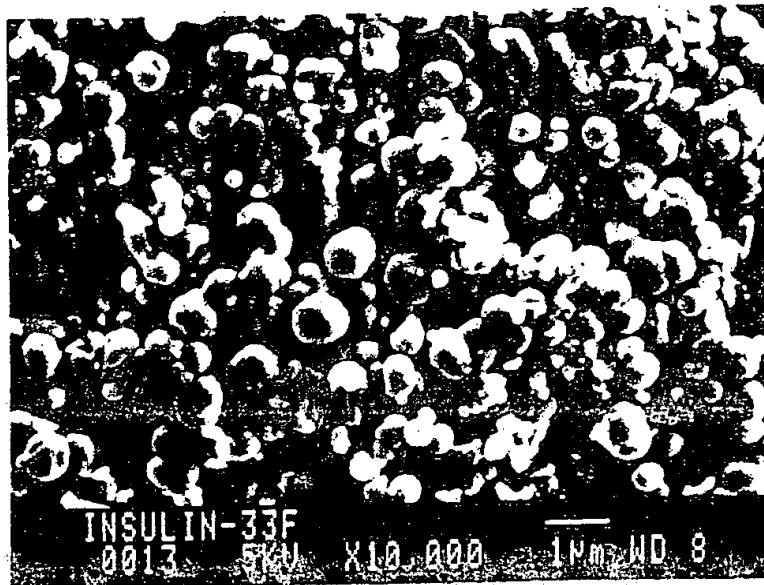


FIG. 6



FIG. 7



DOCUMENTS CONSIDERED TO BE RELEVANT					
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. CL.5)		
X	WO-A-9 003 782 (THE UPJOHN COMPANY) * page 2, line 31 - page 3, line 24 * * claims 2,10-13 *	1	C07K3/00 A61K9/16 C07K3/24		
A	* the whole document *	2-12			
X	CHEMICAL ABSTRACTS, vol. 111, December 1989, Columbus, Ohio, US; abstract no. 216768q, GALLAGHER, P.M. ET AL. 'Gas antisolvent recrystallization: new process to recrystallize compounds insoluble in supercritical fluids.' page 141 ;column 2 ; * abstract *	1			
A		2-12			
D	& ACS Symp.Ser. 1989, 406, 334-54.				
TECHNICAL FIELDS SEARCHED (Int. CL.5)					
A61K C07K					
The present search report has been drawn up for all claims					
Place of search	Date of completion of the search	Examiner			
BERLIN	16 FEBRUARY 1993	AVEDIKIAN P.F.			
CATEGORY OF CITED DOCUMENTS					
X : particularly relevant if taken alone	T : theory or principle underlying the invention				
Y : particularly relevant if combined with another document of the same category	E : earlier patent document, not published on, or after the filing date				
A : technological background	D : document cited in the application				
O : non-written disclosure	L : document cited for other reasons				
P : intermediate document	R : member of the same patent family, corresponding document				